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Cellular Growth of Host and Symbiont in a Cnidarian-Zooxanthellar Symbiosis

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Abstract. The hydroid *Myrionema ambionense*, a fast-growing cnidarian (doubling time = 8 days) found in shallow water on tropical back-reefs, lives in symbiosis with symbiotic dinoflagellates of the genus *Symbiodinium* (hereafter also referred to as zooxanthellae). The symbionts live in vacuoles near the base of host digestive cells, whereas unhealthy looking zooxanthellae are generally located closer to the apical end of the host cell. Cytokinesis of zooxanthellae occurred at night, with a peak in number of symbionts with division furrows (mitotic index, MI = 12%–20%) observed at dawn. The MI of zooxanthellae decreased to near zero by the middle of the afternoon and remained there until the middle of the next night. Densities of live zooxanthellae living inside of host digestive cells peaked following cytokinesis, whereas densities of unhealthy looking symbionts were highest just before the division peak. Mitosis of host digestive cells was highest in the evening, also preceding the peak in zooxanthellar MI. This is the first study relating phased host cell division to diel zooxanthellar division in marine cnidarians.

Food vacuoles were prevalent inside of digestive cells of field-collected hydroids within a few hours after sunset and throughout the night, coinciding with digestion of captured demersal plankton. Laboratory experiments showed that food vacuoles appeared in digestive cell cytoplasm within 2 h of feeding with nauplii of *Artemia*. The number and size of food vacuoles per digestive cell and the percentage of digestive cells with food vacuoles all decreased 5–7 h following feeding in laboratory experiments, and by midday in field-collected hydroids.

Light and external food supply were important in maintaining phased division of the symbionts, with a lag in response time to both parameters of 11–36 h. Altering light and feeding during the night did not influence the level of the peak MI the next morning, though in one experiment the absence of light slowed final separation of daughter cells at the end of cytokinesis. In another experiment, hydroids starved for 3–7 d and "pulse-fed" *Artemia* nauplii for 1 h at the beginning of the dark period showed continued low symbiont division (<5%) after 11 h, whether maintained in constant light or darkness, implying that most algal division is set more than 24 h prior to actual cytokinesis. Transferred to a 14:10 h light:dark cycle for another 24 h (36 h after feeding), the same hydroids exhibited a "normal" peak MI (*ca.* 15%) at dawn, but zooxanthellae from hydroids kept in constant darkness still showed a low MI. These results show that mitosis of symbiotic dinoflagellates requires three factors: external food; a minimum period of time following feeding (11–36 h), presumably for digestion; and a period of light following feeding, presumably to provide carbon skeletons necessary for completing cytokinesis.

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Introduction

Although the ecological significance of cnidarian-zooxanthellar symbioses has been recognized for over 70 years (Yonge and Nichols, 1931), efforts to understand how these associations remain together have been sporadic. Most algal symbioses are characterized by relatively constant densities of symbionts (Reimer, 1971; Muscatine and Pool, 1979; McAuley, 1994), giving rise to theories that symbiotic algae are "regulated" by their hosts. The three proposed modes of maintaining densities of symbionts are (1) expulsion or exocytosis of extra symbionts, (2) digestion of extra symbionts, and (3) control of growth of endosymbionts by superimposition of an external control, or by limiting nutrient supply (Muscatine and Pool, 1979). The first two methods assume that "extra" algae are produced by algal division, and that the host has some mechanism of detecting and responding to increased densities of symbionts, somehow

culling supernumerary algae down to the steady-state density. The third method relies on integration of host and symbiont division cycles. Both expulsion and synchronized division are present in most cnidarian-algal symbioses that have been investigated (refs. below), but little evidence exists for digestion of extra symbiotic algae.

The approach taken in this study is patterned after the productive research conducted on hydra-zoochlorellae symbioses. Green algae in the genus *Chlorella* live symbiotically in vacuoles at the base of host digestive cells in some types of *Hydra*, and this symbiosis has been widely used as a model system. In *Hydra*, algal volume per digestive cell is positively correlated with size of the host cell, suggesting that available space is a factor in determining the symbiont population size (Douglas and Smith, 1984). Zoochlorellae divide synchronously within the host cell following host feeding (McAuley, 1982, 1985, 1986); mitosis of digestive cells and their symbiotic algae increases about 12 h after feeding (McAuley, 1982). However, the number of zoochlorellae within dividing host cells increases before the host cells complete cytokinesis (McAuley, 1982, 1986), suggesting that in normal culture conditions zoochlorellae often divide before the host cells do. A similar phenomenon occurs when hydra regenerate (McAuley, 1986). To grow and survive in hydra, zoochlorellae require light as well as food, and their numbers are reduced in animals kept in the dark (Pardy, 1974a, b).

There are few analogous studies on control of cellular proliferation of symbiotic dinoflagellates in marine cnidarian host cells. However, many studies have documented the dissociation of these symbioses; these have largely focused on recent coral "bleaching events," which involve the loss of algal symbionts or their pigments (see references in Jokiel and Coles, 1990). Research in this area indicates that each partner in the symbiosis has its own physiological requirements and tolerances, and that even subtle changes in factors influencing the physiology of either partner may radically alter the steady-state of the symbiosis (*i.e.*, Porter *et al.*, 1989; Iglesias-Prieto *et al.*, 1992; Gates *et al.*, 1992; Fitt *et al.*, 1993, 1995).

Symbiotic dinoflagellates typically show peaks of dividing cells at dawn or at the beginning of the light period. For instance, cultured zooxanthellae maintained on a 14:10 light:dark cycle show division peaks at the beginning of the light period; the peaks are followed by the production of motile cells (Fitt and Trench, 1983). Zooxanthellae living in host gastrodermal cells (Muscastine *et al.*, 1998) exhibit phased division inside of the jellyfish *Mastigias* sp. (Wilkerson *et al.*, 1983), the hydroid *Myrionema amboinense* (Michael and Fitt, 1984; Fitt and Cook, 1990; McAuley and Cook, 1994), the sea anemone *Aiptasia pallida* (Cook *et al.*, 1988), and five species of Indo-Pacific reef corals (Smith and Hoegh-Guldberg, 1987; Hoegh-Guldberg, 1994). In contrast, asynchronous division of symbionts was reported from nine species of reef corals from Discovery Bay, Jamaica (Wilkerson *et al.*, 1988). Studies investigating synchrony of zooxanthellar mitosis with host cell division are generally lacking; limited data from the Caribbean staghorn coral *Acropora cervicornis* indicate night-time peaks in host cell division (Gladfelter, 1983).

The basis for the diel division patterns seen in zooxanthellar symbioses is not clear. It has been suggested that diel cycling of intracellular pH, driven by photosynthetic utilization of intracellular carbon dioxide, may be responsible by providing pulses of diffusible ammonia/ammonium (Fitt *et al.*, 1995). Pulses of nitrogen have long been thought responsible for phased division of phytoplankton in nature (*i.e.*, Doyle and Poore, 1974), and additions of high concentrations of dissolved nitrogen to seawater damped out the diel rhythm of zooxanthellar division in *Pocillopora damicornis* (Hoegh-Guldberg, 1994). That nutrients, either dissolved or from external food, are involved in the division of host cells and zooxanthellae is neither surprising nor as interesting as the temporal relationships between host feeding, availability of nutrients to symbionts, and mitosis of host cells and their intracellular zooxanthellae.

The tropical shallow-water marine hydroid *Myrionema ambionense* shares several characteristics with the green hydra symbiosis, making it a good model system for cellular studies of marine dinoflagellate symbioses: it has relatively rapid growth, it can be maintained in the laboratory, and the dynamics of host and symbiont cell relationships can be analyzed with cell maceration techniques. This study relates natural diel patterns of zooxanthellar division inside of hydroid host cells to diurnal feeding of the host, host cell division, and exposure to natural light:dark cycles.

Materials and Methods

Collection and maintenance of animals

Colonies of the hydroid *Myrionema ambionense* were collected from shallow-water (< 2 m) habitats adjacent to the Discovery Bay Marine Laboratory in Jamaica and used immediately in experiments. In some experiments animals were maintained in glass petri dishes in the laboratory in unfiltered seawater (SW) obtained from the laboratory seawater system at ambient air and water temperature (26°–28°C) and light (*ca.* 80 $\mu E\ m^{-2}s^{-1}$).

Determination of mitotic index (MI), symbiont densities, and vacuoles

Unless indicated otherwise, all experiments involved macerating (David, 1973) 5–10 polyps from each of six colonies of hydroids, each polyp including < 2 mm of stolon. Zooxanthellae from 100 digestive cells were observed and the number of symbionts in each cell was counted. Unhealthy looking zooxanthellae in host cells were

counted from the same 100 digestive cells. The unhealthy looking dinoflagellaes were distinguished from their live counterparts by their lack of circular symmetry and relatively uneven and darker coloration (see Fig. 1C). The percentage of zooxanthellae dividing (mitotic index = MI) was determined from microscopic counts of cells with division furrows (doublets). The mean percentage of dividing zooxanthellae from each colony of hydroids was calculated from a minimum total of 1000 zooxanthellae. To determine the time of peak division of zooxanthellae in *Myrionema amboinense*, hydroids from each of the six colonies were collected from the field every 3 h for 24 h, macerated within 15 min of collection, and the MI determined.

The volume of the host cells was determined from their depth and surface area as described in Douglas and Smith (1984). Macerated cells are flattened and not cylindrical; they are variable in length and width but usually have a relatively straight basal edge and rounded apex. The distance between the points at which the upper and lower surfaces of the cell just go out of focus was found to be 9–12 μm, as determined from the scale on the focusing knob of the microscope, so the depth of digestive cells in macerations was taken as 10 μm. Surface areas were determined from length and width (average of widths at top and bottom of cell), measured for rectangular hydranth cells, and diameter for circular tentacle cells. Host cell division was determined by staining mitotic figures of digestive cells in macerated preparations with 4′,6-diamidino-2-phenylindole (DAPI) (Falkowski and Owens, 1982; McAuley, 1982). Number and estimated volume of intracellular vacuoles were also determined from the same 100 digestive cells described above. Only relatively large (>2 μm in diameter) vacuoles were monitored. The number and volume of the vacuoles was compared to the volume of the host cell in one experiment to estimate the relative portion of the cell taken up by vacuoles.

Hydroid growth rate

Five days before the beginning of the growth experiment, colonies of *Myrionema ambionense*, with 2 to 15 polyps, were collected and placed on microscope slides in glass petri dishes. Only colonies that had attached to the slides were subsequently used in experiments. Slides with attached hydroids were placed in SW in glass petri dishes in the laboratory or among natural colonies of hydroids in the field. Animals maintained in the laboratory were fed nauplii of *Artemia*, and the water was changed daily. The polyps were counted and the lengths of the stolons were measured for all experimental animals at the start of the experiment and after 7 days.

Factors influencing mitotic index

The relationship of light and feeding to division of symbiotic dinoflagellates was investigated in two experiments. In the first experiment hydroids were collected from the field in the dark just after dusk or preceding dawn. Half of the hydroids collected at each time were kept in constant dark, the other half in constant light (*ca.* 80 μE m^{-2}s^{-1}). The mitotic index of the zooxanthellae was determined from macerated hydroids, as described above, every 1–2 h for about 16 h after collection.

In the second experiment hydroids were unfed for 3–7 days and then fed *Artemia* nauplii for 1 h at the beginning of the dark period; they were then removed from the food source. Half the animals were subsequently maintained in constant light, the other half in the dark. At dawn (*i.e.*, 11 h following feeding) the MI of the symbionts was determined. Hydroids maintained in the dark were divided into two groups again, and held another 24 h in either a 14:10 h light:dark cycle or in constant dark. The MI was determined again at the next dawn, about 36 h after feeding.

Results

Distribution of zooxanthellae

Zooanthellate digestive cells of *Myrionema ambionense* have two general shapes: columnar from the hypostome (= cup) region of the hydranth (also called digestive cells here, Fig. 1) and circular from the tentacle portion of the hydranth (Fig. 2). The circular form is donut-shaped with a hole where the coelenteron extends up each tentacle (Fig. 2). Intermediate morphologies are found at the base of the tentacles (*i.e.*, Fig. 2c). Healthy looking zooxanthellae were located near the base of host hypostome cells, adjacent to the mesoglea (Fig. 1A–D), and usually at the periphery of tentacle cells, depending on the angle of observation (Fig. 2). Recently fed hydroids maintained in the laboratory also had zooxanthellae at the base of their digestive cells but contained many more vacuoles (Fig. 1B) than seen in unfed hydroids (Fig. 1A). Unhealthy looking zooxanthellae were usually located near the apical end of the cell, between the host nucleus and the coelenteron (Fig. 1C).

Most of the digestive cells in the hypostome of the hydroid contained one, two, or three zooxanthellae; only about a quarter harbored more than three symbionts (Figs. 1, 3). Gastrodermal cells in tentacles appear to develop from digestive cells in the hypostome that migrate from a presumed central division zone. Their bases and cell volume expand as they encircle the inside of the hollow tentacle (Fig. 2C), giving rise to their characteristic circular and semicircular shapes (Fig. 2). In contrast to digestive cells in the hypostome, tentacle cells usually held more than 10 zooxanthellae, with more than 75% of the tentacle cells containing between 10 and 40 zooxanthellae (Fig. 3). The

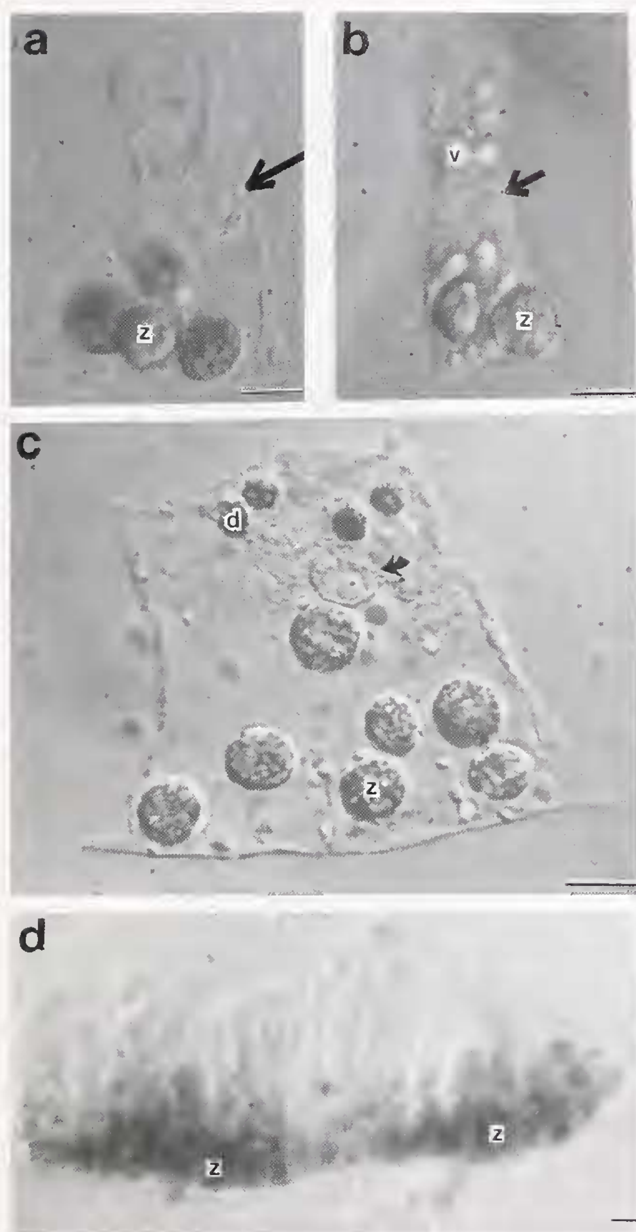

Figure 1. Gastrodermal cells from the hypostome of the hydroid of *Myrionema ambioinense*. (a) Columnar digestive cell from hydroids collected in the afternoon containing four healthy looking zooxanthellae (z) and few vacuoles (v). (b) Columnar digestive cell from hydroids collected at night containing two zooxanthellae and numerous vacuoles. (c) Healthy looking zooxanthellae at basal end of this digestive cell contrast with four degenerate or unhealthy looking zooxanthellae (d) at the distal end of the digestive cell. (d) Row of columnar digestive cells from partially macerated polyp showing zooxanthellae at the base, away from the phagocytic distal end of their host cell. Arrows show centrally located host nucleus with prominent nucleolus. Bars = 10 μm.

highest number of symbionts in a single digestive cell was 69, observed in a tentacle cell.

Estimates of the relative volume of digestive cells in relation to their population of symbionts suggest that zooxanthellae may be limited by available space or physiological parameters associated with the size of the host cell. Larger host cells contained more zooxanthellae than smaller host cells, and the number of zooxanthellae residing inside of a digestive cell was directly correlated with the relative volume of the cell (Fig. 4). The average number of zooxanthellae in each hydroid polyp, including 1–2 mm of stalk, was 2.4 ± 0.2 (mean ± SD) × 10^5 (n = 15 different colonies).

Growth

Growth of a colony of *Myrionema* was surprisingly constant. When maintained in the laboratory for 1 week, hydroids grew an average of 4.3 ± 0.8 mm stolon length/d (n = 15) for each piece of stolon. There was no correlation between growth rate and initial size of the colony (range 1–15 polyps). Both laboratory-maintained and field-monitored colonies roughly doubled their number of polyps over an 8-day period (Fig. 5), regardless of colony size.

Diel patterns of host and symbiont division

Zooxanthellate division (MI = 16.6 ± 1.5%, n = 6) from field-collected hydroids peaked at dawn, declining to near zero in the early afternoon and evening (Fig. 6C). Thirty-four percent of the host cells containing zooxanthellae held at least one dividing algal cell over the diel period. The mean density of zooxanthellae per hydranth cell was 2.67. The density of zooxanthellae per hypostome digestive cell was highest in the mid-afternoon following the division peak (*ca.* 2.9 ± 0.1 zooxanthellae per host cell, n = 6), and lowest about 3 h after sunset (*ca.* 2.4 ± 0.4 zooxanthellae per host cell, n = 6) (Fig. 6C). This implies an average growth rate of about 0.5 zooxanthellae per digestive cell per day. The same value is obtained by multiplying the maximum number of zooxanthellae per host cell (2.9) by the MI (16.6%), again resulting in an increase of about 0.5 zooxanthellae per host cell per day. In other words, both methods show that the number of zooxanthellae in each hydranth digestive cell doubles about every 6 days.

The number of unhealthy looking zooxanthellae also exhibited a diel cycle (Fig. 6B). The number of unhealthy looking zooxanthellae per hydranth digestive cell showed a broad peak at about midnight (four highest data points = 1.04 ± 0.14 unhealthy looking zooxanthellae/cell), and reached a broad low during the day (four lowest data points = 0.72 ± 0.05 dead zooxanthellae/cell). To balance the average growth rate of colonies (doubling time = 8 days) with the average growth rate in number of zooxanthellae (doubling time = 6 days), an average digestive cell would have to lose about 1 zooxanthella every 8 days (0.14 zooxanthellae per day). This rate of loss of zooxanthellae is about half that calculated from the difference in average

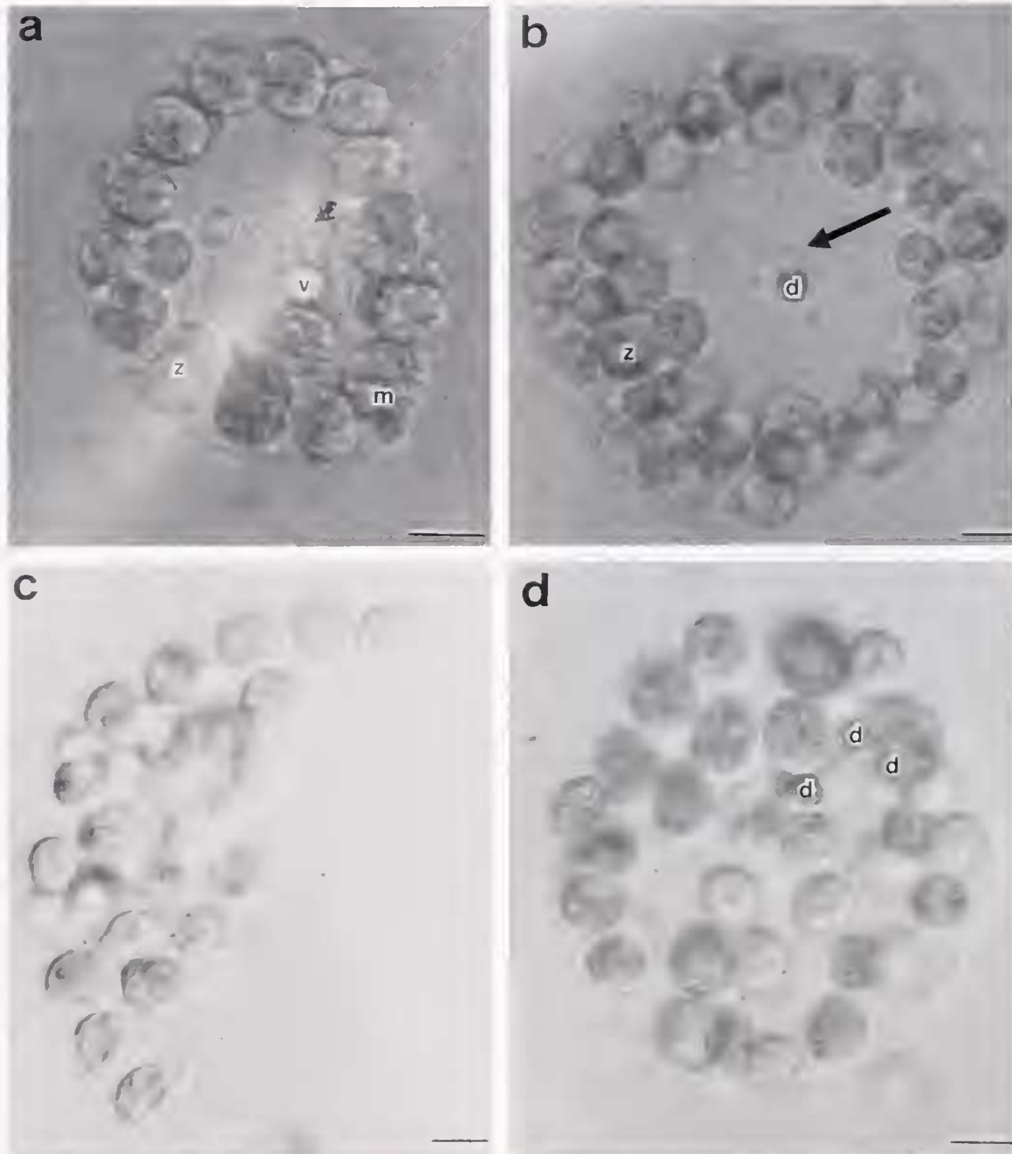

Figure 2. Gastrodermal cells from the tentacles of the hydroid *Myrionema amboinense.* (a, b) Circular disc-shaped digestive cells showing zooxanthellae (z) in the basal portion of the cell which would normally lie adjacent to the mesoglea in the tentacle, host nucleus (arrows) near the center, and lumen (extension of coelenteron) of hollow tentacle immediately below arrow. (c) Half-moon-shaped digestive cell, wrapping around the base of a tentacle. (d) Disc-shaped digestive cell showing degenerate or unhealthy looking zooxanthellae (d) near the center and at one side where the two ends of the base of the cell presumably have met. m = zooxanthella with division furrow indicating cytokinesis at the end of mitosis; v = vacuoles. Arrows show centrally located host nucleus with prominent nucleolus. Bars = 10 μm.

density of dead or moribund-looking zooxanthellae (0.32 zooxanthellae per day) observed over a 24-h period.

Though it was difficult to see final cytokinesis (telophase) of host digestive cells, nuclear staining with DAPI showed that mitosis in these cells (pro-, meta-, and anaphase) occurred predominantly in the evening (peak MI = 2.4 $\pm$ 1.3%, $n = 6$ colonies), compared to other times during the day (MI $< 1\%$) (Fig. 6A). These data suggest that cytokinesis (cell separation) occurs first in the early evening, corresponding with the observed decrease in zooxanthellar density per host cell (see above).

Evidence for diel host feeding

Hydroids collected at night and macerated immediately after collection inevitably contained ingested prey items,

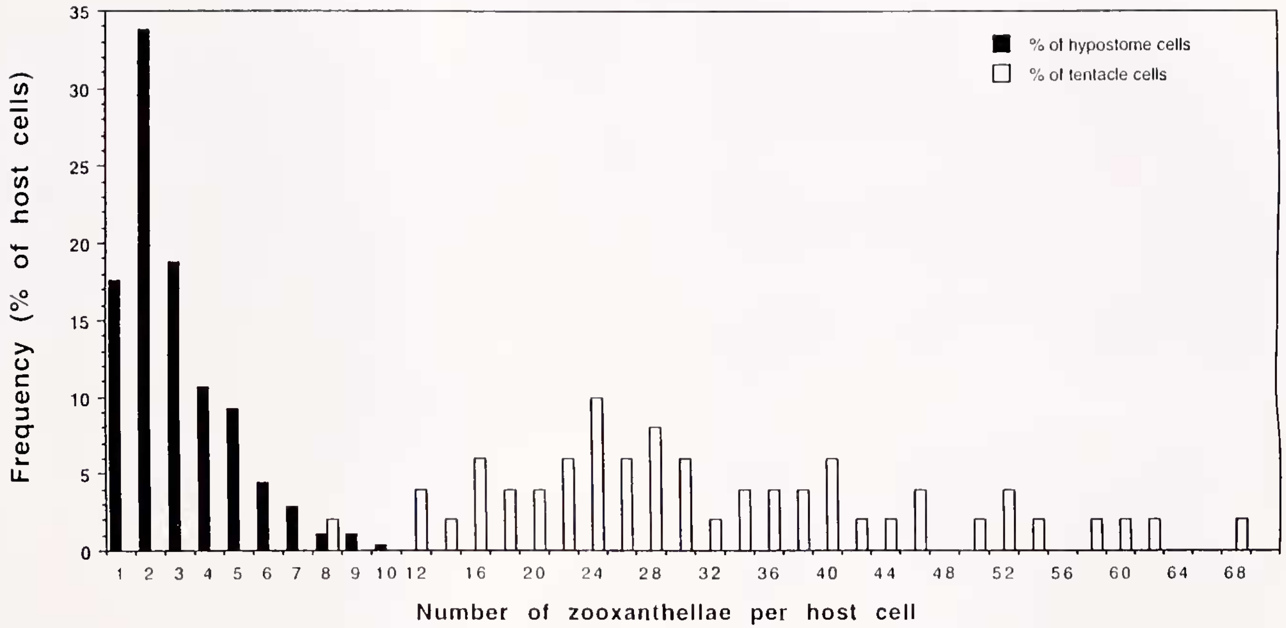

Figure 3. Frequency distribution of host gastrodermal cells of the hydroid *Myrionema ambionense* in relation to density of symbiotic dinoflagellates residing within. Solid bars indicate percent of columnar hypostome cells; clear bars show percent of circular tentacle cells.

such as arthropod exoskeletons, setae, and unidentified debris, that were not present in hydroids collected during the day. In addition, the presence of vacuoles within digestive cells showed a prominent diel cycle, with a rapid rise immediately after dusk in the percentage of digestive cells containing vacuoles and the number of vacuoles per digestive cell (Fig. 6A). Maximum values for these parameters extended from the middle of the night to the morning, then slowly decreased during the morning hours to afternoon lows. This pattern suggested that the vacuoles are actually food vacuoles associated with nighttime feeding by the hydroids. To test this hypothesis, starved animals (3–7 days) were fed brine shrimp nauplii for 1 h in the laboratory, then examined for vacuole formation hourly for the next 13 h. Within 2 h of feeding the percentage of host cells with vacuoles, the number of vacuoles per host cell, and the relative volume of the host cell filled with vacuoles in-

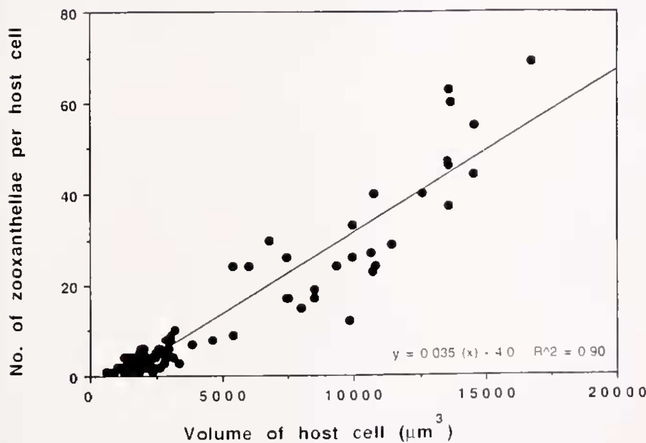

Figure 4. Relationship between number of symbiotic dinoflagellates and the volumn of the host (*Myrionema ambionense*) gastrodermal cells they live in.

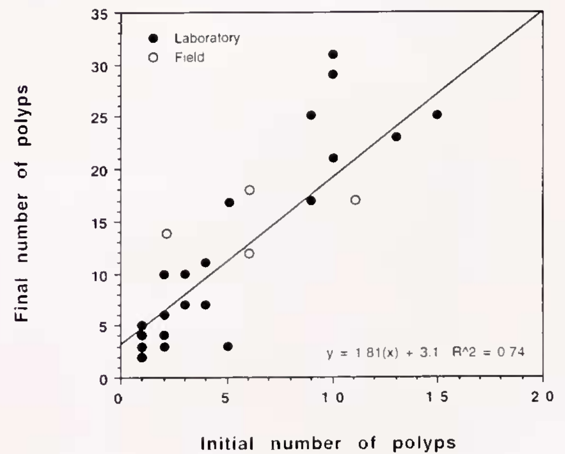

Figure 5. Growth after seven days of different-sized colonies of *Myrionema ambionense* in relation to initial colony size (1–15 polyps). Colonies were attached to glass slides and monitored both in the laboratory and the field.

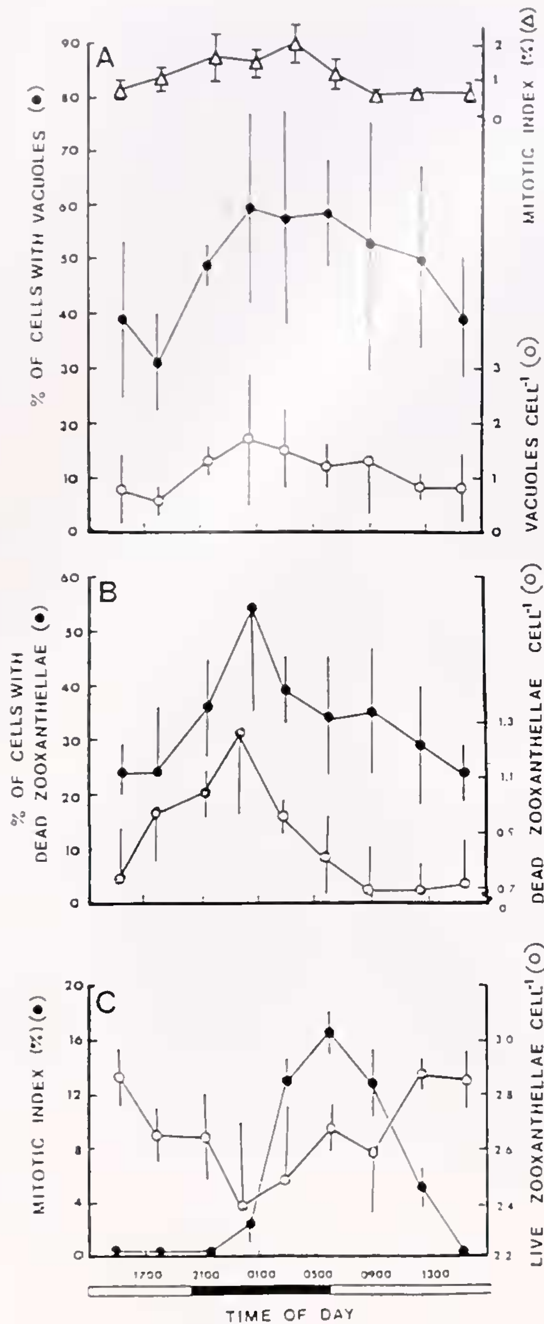

Figure 6. Distribution of symbiotic dinoflagellates (zooxanthellae) and food vacuoles within individual host cells of *Myrionema amboinense* in relation to time of day collected from the field. (A) Percent of hypostome digestive cells containing vacuoles and number of vacuoles per digestive cell and host cell mitotic index. (B) Percent of hypostome digestive cells containing degenerate or unhealthy looking zooxanthellae and number per digestive cell. (C) Percent of zooxanthellae with division furrows (mitotic index) and number of healthy looking zooxanthellae per hydranth digestive cell. Data presented as mean ± SD, $n = 6$. Dark bar on x axis denotes night; open bar indicates daytime.

creased rapidly (Fig. 7). These parameters returned to prefeeding levels about 6–8 h after feeding.

Environmental factors and relationship to diel patterns

The influence of photoperiod and feeding on zooxanthellate division was determined in two experiments. In the first experiment (Fig. 8A), hydroids collected at dusk and maintained without food in either constant light or dark showed the same level and timing of MI as seen in hydroids in the field (Fig. 6). Thus it appears that altering food and the light and dark periods less than 12 h before the expected peak in MI does not influence the division patterns of the zooxanthellae.

Hydroids that were collected from the field at the *end* of the dark period (when MI of symbionts was highest) and maintained in constant light or in constant dark showed

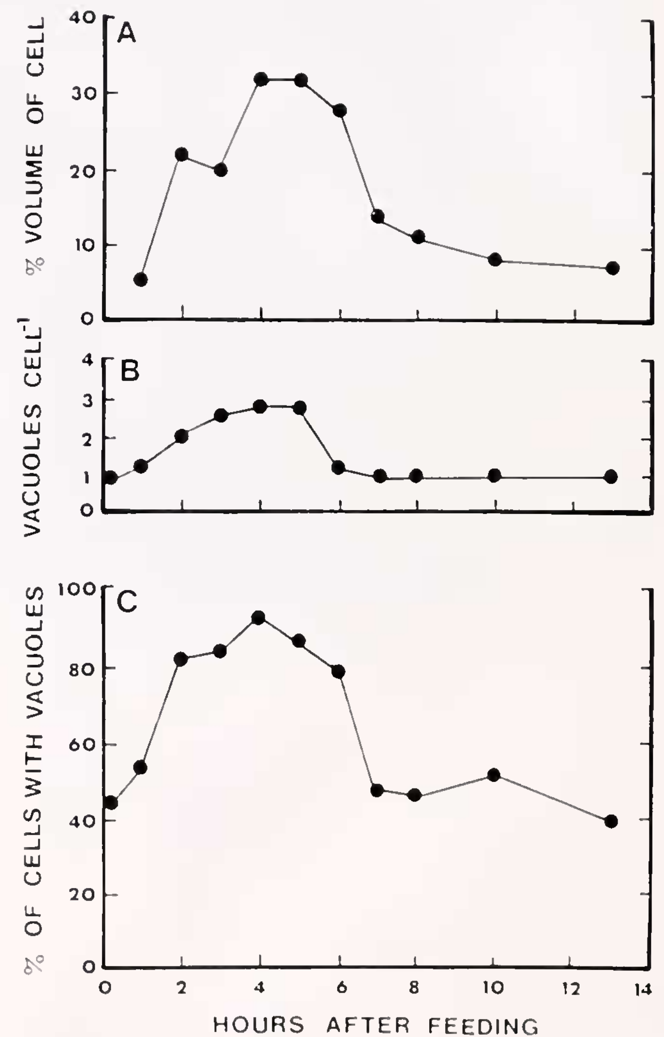

Figure 7. Relationship between vacuole appearance in digestive cells of *Myrionema amboinense* following a 1-h pulse-feeding with nauplii of brine shrimp (time 0). (A) Volume (area) of host cells occupied by vacuoles. (B) Average number of vacuoles per cell. (C) Percentage of digestive cells containing vacuoles. Each data point is the mean of 100 digestive cells from the hypostome.

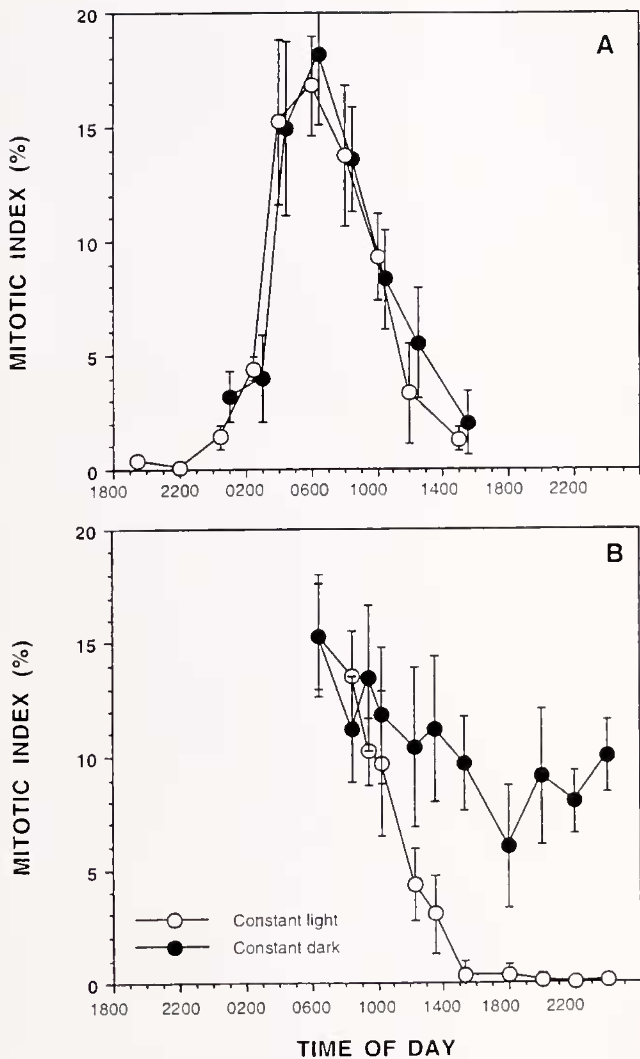

Figure 8. Relationship between mitotic index of symbiotic dinoflagellates residing in digestive cells of *Myrionema ambionense* when hydroids were either (A) collected at dusk (1900) and maintained in either constant light (open symbols) or constant dark (closed symbols) or (B) collected at the end of the night and maintained similarly in either constant light or dark.

slightly different results (Fig. 8B). Zooxanthellae from hydroids maintained in the light showed no changes in the division patterns seen in the field. In contrast, the hydroids maintained in constant darkness showed a slower decline in zooxanthellar cytokinesis during the afternoon period (Fig. 8B), suggesting that light was critical for the final stages of symbiont cytokinesis in this experiment.

In the second experiment, starved hydroids (3–7 days) were pulse-fed for an hour at the beginning of the dark period in the laboratory, then either maintained in constant light or left in the dark (Fig. 9). About 12 h after the pulse-feeding, those hydroids kept under constant light during the night had a higher MI (4.2% ± 1.4% SD, n = 6) than those hydroids kept in the dark (1.6% ± 0.9% SD, n = 6). The hydroids maintained in the dark for 12 h were then divided into two groups. The group that remained in the dark for an additional 24 h continued to show low algal MI (2.1%), while those moved into a 14:10 h light:dark cycle for 24 h showed a normal level of dividing cells (*ca.* 14.2%) 36 h after feeding. These results show that normal mitosis of symbiotic dinoflagellates in this hydroid requires three factors: external food; a minimum period of time following feeding (11–36 h), presumably for digestion to occur; and a period of light following feeding, presumably to provide carbon skeletons necessary for completing cytokinesis.

Discussion

This study shows that division of zooxanthellae and host cell is synchronized in the marine hydroid *Myrionema ambionense*, and that host feeding and light play an important role in the phased division patterns observed. Most, if not all, zooxanthellae symbioses appear to exhibit phased sym-

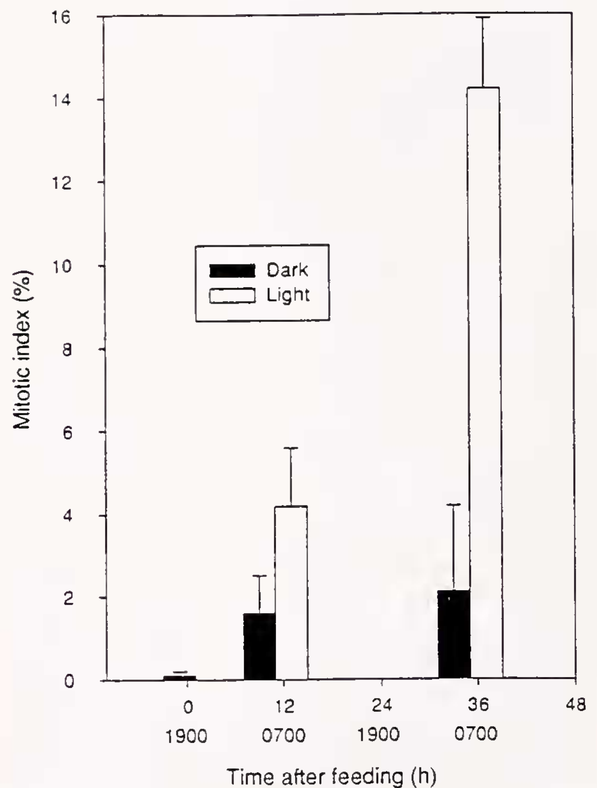

Figure 9. Relationship between feeding, light, and darkness on division (mitotic index) of symbiotic dinoflagellates residing in the hydroid *Myrionema ambionense*. Unfed (3–7 days) hydroids were fed for 1 h at time 0 (1900) and maintained for the next 11 h (12 h after feeding) in either constant dark (filled bars) or light (open bars). Animals kept in the light were then discarded, and those from the dark were maintained an additional 24 h (36 h after feeding) in either constant dark or on a normal 14:10 h light:dark cycle.

biont division *in situ*, with peaks in doublet cells seen around dawn (Michael and Fitt, 1984; Smith and Hoegh-Guldberg, 1987; Fitt and Cook, 1990; Cook and Fitt, 1990; Hoegh-Guldberg and Smith, 1989; McAuley and Cook, 1994; Hoegh-Guldberg, 1994). Symbiotic dinoflagellates in hosts removed from the reef or placed in seawater containing high concentrations of dissolved inorganic nitrogen (DIN) lose their division synchrony. For instance, additions of DIN damped out natural diel patterns of zooxanthellar division in the coral *Pocillopora damicornis* in Hawaii (Hoegh-Guldberg, 1994), and maintenance of the zooxanthellate jellyfish *Mastigias* sp. in the laboratory altered the MI of its symbionts (Muscatine *et al.*, 1986). Perhaps these observations explain asynchronous division of symbionts in nine species of reef corals removed from the reef in Jamaica and maintained in the laboratory in seawater containing high concentrations of DIN (D'Elia *et al.*, 1981; Wilkerson *et al.*, 1988).

This is the first study relating host cell division to zooxanthellar division in marine cnidarians. Research on symbiotic *Hydra* showed peaks in host cell mitosis and division of their *Chlorella* symbionts (cytokinesis) 10–12 h following feeding, such that brief increases in algal density observed before completion of host cell cytokinesis returned the number of symbionts per host cell to a steady-state level (Muscatine and Neckelmann, 1981; McAuley, 1982, 1986). Data from *Myrionema ambionense* suggest that dividing host cells completed cytokinesis *before* their symbiotic dinoflagellates finished dividing, such that *decreases* in symbiont densities were observed in the late afternoon and evening. Early morning cytokinesis of zooxanthellae returned densities of symbionts to their afternoon peak levels. Phased division of endothelial and calicoblastic epithelial coral cells in *Acropora cervicornis* also occurred during the middle of the night, and MI was less than 2% (Gladfelter, 1983). The peak percentage of cells containing mitotic figures in *Myrionema ambionense* was also low compared to the proportion of zooxanthellae dividing daily, due to the likelihood that during the cell cycle mitosis lasts much less than the 3-h sample interval, suggesting that many such events were missed between sample times. For instance, mitosis in well-fed *Hydra* lasted only about 1.5 h (David and Campbell, 1972).

Feeding and light are two factors linked to phased mitosis of zooxanthellae and host digestive cells in *Myrionema ambionense*. Digestive cells in *Hydra* also exhibit a diel periodicity in the mitotic index, with a midnight peak following a daily feeding regime at 1000 h each morning (see references in McAuley, 1994). Similarly, normal feeding of tropical marine cnidarians in nature appears to occur on a diel cycle set by the availability of demersal plankton food (Johannes and Tepley, 1974; Porter, 1974; Alldredge and King, 1977) or alternate sources of nitrogen (see Fitt *et al.*, 1995). Demersal plankton are thought to be most abundant on the reef at dusk and dawn (Glynn, 1973), though densities throughout the night are at least an order of magnitude greater than daytime densities. The data presented in the present study suggest that *Myrionema ambionense* feeds on demersal plankton at night, as evidenced by increased numbers of vacuoles in digestive cells following dusk in field populations of hydroids, and by laboratory feeding experiments (Fig. 7). Feeding on zooplankton provides nitrogen needed for cell growth, and night-time feeding would provide regular diel pulses of nitrogen. Nitrogen pulses are known to influence the timing and amount of algal division (Doyle and Poore, 1974). For instance, some diatoms maintained in the laboratory on a light-dark cycle, or in constant light, exhibited peaks of cell division following a pulse of nitrogen, whereas additions of the same daily total of nitrogen in equal increments throughout the day eliminated the periodicity within 24 h (Quarmby *et al.*, 1982; Yoder *et al.*, 1982). In giant clams, diffusible ammonia/ammonium in seawater is available at night, due to changes in pH within the host, driven by symbiont photosynthesis (Fitt *et al.*, 1995). A similar mechanism of delivering pulses of nitrogen to symbiotic reef corals and other cnidarians may control phased mitosis of their symbiotic dinoflagellates. Indirect support for this hypothesis includes the interesting observation that continuous addition of high concentrations of dissolved nitrogen to the coral *Pocillopora damicornis* caused a damping of diel peaks in MI of their zooxanthellae (Hoegh-Guldberg, 1994).

Light is also required for mitosis of symbiotic dinoflagellates, most likely to provide carbon skeletons needed both for the assimilation of DIN as well as for the respiratory metabolism associated with completion of cytokinesis, as evidenced by damping of the division patterns of zooxanthellae when the normal light:dark period is disturbed (Figs. 8b, 9). The intriguing delay in algal mitosis following feeding of starved hosts (Fig. 9) involves light, and is also seen in green hydra, where peaks in algal MI follow those of the host cells by 12 to 24 h (McAuley, 1985). The results for both symbioses suggest that although host cell mitosis follows host feeding by 12 to 24 h, the algal symbiont cell cycle is set 12 to 24 h later (24–36 h following feeding, depending on the synchronization of light and feeding cycles). These results also suggest that the close regulatory relationship between host and symbiont division may be easily disrupted. For instance, the *Chlorella* symbionts in green hydra will overgrow and actually burst their host digestive cell when unfed hydra are placed in a complex mixture of inorganic nutrients in the light (Muscatine and Necklemann, 1981).

Virtually all zooxanthellate cnidarians appear to expel some of their symbiotic dinoflagellates from their coelenteron on a daily basis (*e.g.*, Reimer, 1971; Hoegh-Guldberg *et al.*, 1987), whereas mass expulsion of zooxanthellae has been attributed to severe osmotic or thermal stress

(Goreau, 1964; Jokiel and Coles, 1977, 1990; Jaap, 1979; Glynn, 1983). The released zooxanthellae may be of normal morphology, with dividing and motile cells, or they may appear unhealthy, as described here. Most researchers feel that expulsion from the host is a way to partially balance increases of symbionts from algal division (Reimer, 1971; Hoegh-Guldberg *et al.*, 1987), and in cases where live zooxanthellae are released, a dispersal mechanism for infecting new hosts (Trench, 1979; Muller-Parker, 1984). Many sea anemones (Steele, 1977) and all of the giant clams (Trench *et al.*, 1981) release motile forms of zooxanthellae daily. Hoegh-Guldberg *et al.*, (1987) monitored a fire coral, a reef-building coral, and two soft corals and found that expulsion increased and most often peaked during the first part of the dark period (night). Smith and Muscatine (1986, pers. comm.) found the same pattern in extrusion of algal pellets from the sea anemone *Aiptasia pulchella*, though Stimson and Kinzie (1991) found peaks in release of zooxanthellae from the coral *Pocillopora damicornis* during the day. Unhealthy looking zooxanthellae seen in digestive cells of *Myrionema* are also most prevalent in the middle of the night. Although actual release by the host *in situ* has not been observed, hydroids maintained in the laboratory in petri dishes inevitably had small pellets made up of unhealthy looking zooxanthellae around them in the morning; presumably these were released by the polyps during the previous night. The density of unhealthy looking symbionts in *Myrionema* was negatively correlated with the density of healthy looking symbionts, such that the appearance of new zooxanthellae helped to balance diel loss from the release of unhealthy looking symbionts and from dilution due to host growth.

The results of this study also relate the high MI of symbiotic dinoflagellates to the rapid growth rates seen in *Myrionema ambionense* in the field (Fig. 5). Fire corals of the genus *Millepora* also grow fast and exhibit high rates of symbiont growth (Wilkerson *et al.*, 1988). These observations suggest that many of the "new" algae originating during mitosis (doubling time = 6 days) are in "new" digestive cells of *Myrionema ambionense* during normal growth of the animal (doubling time = 8 days) and are at least partially responsible for the high growth rates of the host. The role of host feeding and starvation in *Myrionema ambionense*, and the effects of additions of dissolved inorganic nutrients on zooxanthellar division patterns, as well as the role of light in the natural habitat, are investigated further in companion papers (Fitt and Cook, unpubl.). The ease of experimental manipulation, the availability of replicate polyps that can be collected with little or no damage to the reef, and the ability to clearly visualize zooxanthellae in host cells make this zooxanthellar symbiosis potentially amenable to answering some of the outstanding questions in marine symbioses.

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